output voltage, which is proportional to force, is amplified, scaled, and displayed on a digital voltmeter calibrated directly in kilograms. The display on the digital panel meter is held at the maximum force value by utilizing the tablet breakage point sensing circuitry in the hardness tester.

Calibration and linearity of the digital readings can be checked by displacing the anvil on the hardness tester in small increments while noting the reading on the meter incorporated in the hardness tester. Magnet placement, Hall device power supply voltage, and scaling amplifiers are adjusted for the proper reading.

A method for calibrating the hardness tester was mentioned previously (2).

Advantages of the digital approach include: *(a)* accuracy, *(b)* low cost of the transducer and system, *(c)* ease of construction, *(d)* no change in operating procedures, *(e)* automatic printout if desired, and *(f)* hardness tester not affected by operation of the transducer.

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Drug–Nitrite Interactions: Formation of *N*-Nitroso, C-Nitroso, and Nitro Compounds from Sodium Nitrite and Various Drugs under Physiological Conditions

Keyphrases □ Nitrite–drug interactions—formation of *N*-nitroso, C-nitroso, and nitro compounds from sodium nitrite and 20 drugs under physiological conditions □ Nitroso compounds—formation from sodium nitrite and 20 orally administered drugs under physiological conditions □ Interactions—20 orally administered drugs with sodium nitrite, formation of *N*-nitroso, C-nitroso, and nitro compounds under physiological conditions

To the Editor:

Many alkyl, aryl, and cyclic *N*-nitroso compounds are known to be carcinogenic (1, 2). Recently, several drugs containing secondary and tertiary amino groups were found to react with sodium nitrite *in vitro* and *in vivo* to yield *N*-nitroso compounds (3–8). Coadministration of aminopyrine and sodium nitrite by the oral route produced liver tumors in rats identical to those produced by *N*-nitrosodimethylamine, a product of drug–nitrite interaction in the stomach (9, 10). Similarly, concurrent administration of piperazine and sodium nitrite to mice induced lung adenomas attributable to *N*-nitrosopiperazine formed *in vivo* (11).

Since many orally administered drugs contain secondary and tertiary amino groups which could form potentially toxic *N*-nitroso compounds in the stomach, where suitable acidic conditions and significant concentrations of nitrite through dietary intake may prevail, the drug–nitrite interaction has been implicated in the etiology of human cancer (2, 12–15).

In the present communication, the isolation and mass spectrometric identification of *N*-nitroso, C-nitroso, and nitro compounds that arise from the reaction of sodium nitrite with 20 drugs (Table I) are described. These drugs were selected because they are commonly administered by the oral route and represent a wide variety of chemical structures. These studies were initiated to gain a better understanding of drug–nitrite interactions and to delineate the structural features of the drugs most likely to yield potentially toxic nitroso compounds.

The nitrosative cleavage of tertiary amines was studied previously (16–18). We observed that, depending upon the structure of drugs, five types of nitroso compounds may be formed as a result of drug–nitrite interactions (Table I). For the reactions at pH 1–2, 100 mg of drug was incubated with 200 mg of sodium nitrite in 10 ml of 10% hydrochloric acid at 37° for 4 hr; the reactions at pH 3–4 were carried out in 10 ml of 10% acetic acid. The reaction mixtures were adjusted to pH 11 with solid sodium hydroxide and extracted twice with 8 ml of methylene chloride and twice with 8 ml of ethyl acetate.

The volatile *N*-nitroso compounds in the methylene chloride extracts were identified by GC–mass spectrometry and quantitated by GC (19). The nonvolatile nitroso and nitro compounds in the ethyl acetate extract were purified by TLC on silica gel, using a solvent system of ethyl acetate–methanol (1:1), and characterized by mass spectrometry. The yields were calculated on the basis of the isolation of homogeneous products (8, 20). The methylene chloride extracts contained the products listed in Groups 1 and 2, while the ethyl acetate extracts contained the products in Groups 3–5.

The mass spectra of all *N*-nitroso compounds in Groups 1 and 2 (Table I) were consistent with those reported in the literature (19, 21). The *N*-nitroso derivatives in Groups 3 and 4 exhibited fragmentation patterns typical of *N*-nitroso compounds (the characteristic loss of NO from the parent ion) as well as those consistent with the drug structure (8, 20). The mass spectra of synthetic 4-nitrosoantipyrine and 4-nitroantipyrine (22) were identical to the spectra of the respective products from the reaction of antipyrine (XX) with nitrite.

The following comments may be made regarding the formation of nitroso and nitro compounds from drug–nitrite interactions.

Group 1 Products (*N*-Nitrosodialkylamines)—

Table I—Nitroso and Nitro Compounds from the Reaction of Sodium Nitrite with Orally Administered Drugs

Number	Drug	Nitroso or Nitro Compound Formed	Yield[a], % Reaction at pH 1–2	Yield[a], % Reaction at pH 3–4
		Group 1 Products		
I	Aminopyrine[b]	*N*-Nitrosodimethylamine	31.4	71.4
II	Captodiame	*N*-Nitrosodimethylamine	0	0.7
III	Chlophedianol	*N*-Nitrosodimethylamine	0	0.3
IV	Chlorpromazine[b]	*N*-Nitrosodimethylamine	0	0.6
V	Fonazine	*N*-Nitrosodimethylamine	0	0.8
VI	Oxytetracycline[b]	*N*-Nitrosodimethylamine	0.6	63.2
VII	Diethazine	*N*-Nitrosodiethylamine	0	0.4
VIII	Lucanthone[b]	*N*-Nitrosodiethylamine	0	0.5
IX	Thiphenamil	*N*-Nitrosodiethylamine	0	0.3
		Group 2 Products		
X	Clemizole	*N*-Nitrosopyrrolidine	0	1.4
XI	Cycrimine	*N*-Nitrosopiperidine	0	1.8
XII	Hexadiphane	*N*-Nitrosohexamethyleneimine	0	2.1
XIII	Phenadoxone	*N*-Nitrosomorpholine	0	2.5
XIV	Butaperazine	*N*,*N*′-Dinitrosopiperazine	0	2.8
		Group 3 Products		
IV	Chlorpromazine	*N*-Nitrosodesmethylchlorpromazine	0.4	1.7
XV	Imipramine	*N*-Nitrosodesipramine	0.3	1.2
XVI	Tripelennamine	*N*-Nitrosodesmethyltripelennamine[c]	0.7	5.8
		Group 4 Products		
XVII	Desipramine	*N*-Nitrosodesipramine	10.9	2.8
XVIII	Piperazine[b]	*N*-Nitrosopiperazine	35.0[d]	54.6[d]
		N,*N*′-Dinitrosopiperazine	78.4	84.6
XIX	Protriptyline	*N*-Nitrosoprotriptyline	11.6	3.4
		Group 5 Products[e]		
XX	Antipyrine	4-Nitrosoantipyrine	0[f]	60.8[f]
		4-Nitroantipyrine	89.3	83.7

[a] Percentage of theoretical yield from the reaction of drug with sodium nitrite at 37° for 4 hr. [b] Reaction of this drug with nitrite was reported earlier (3, 5, 24, 31). [c] Formation of this *N*-nitroso derivative was described recently (8). [d] Yield of the mononitroso derivative when the reaction was run for 15 min. [e] Aromatic mononitro and dinitro derivatives of IV, XV, XVII, and XIX were formed (yield of 1–5%) during the reaction of these drugs with nitrite at pH 1–2. Studies are in progress to deduce the structure of these nitro compounds. [f] Yield of the nitroso compound from the reaction at room temperature. Nearly quantitative yield of 4-nitrosoantipyrine can be achieved if the reaction is run in 10% HCl at 0° (22).

The nitrosative cleavage of tertiary amino groups from the dialkylaminoalkyl side chain in Compounds II–V and VII–IX appeared to be the least favored reaction and was completely inhibited at pH 1–2. However, such cleavage of tertiary amino groups attached to ring structures (I and VI) occurred quite readily; indeed the yield of *N*-nitrosodimethylamine formed from aminopyrine was quite high (31.4%) even at pH 1–2.

Group 2 Products (*N*-Nitroso Cyclic Amines)—The yield of cyclic *N*-nitroso compounds from the drug–nitrite reaction at pH 3–4 was between 1.4 and 2.8% with Compounds X–XIV, which contain pyrrolidine, piperidine, hexamethyleneimine, morpholine, and piperazine moieties. Group 2 products were not found when the reactions were carried out at pH 1–2.

Group 3 Products (*N*-Nitroso Derivatives of *N*-Dealkylated Drugs)—Between the two pH ranges examined, higher yields of *N*-nitroso derivatives were obtained at pH 3–4 with all three drugs (IV, XV, and XVI) that gave Group 3 products with nitrite.

Group 4 Products (*N*-Nitroso Derivatives of Drugs)—The reaction of nitrite with drugs containing secondary amino groups yielded substantial quantities of *N*-nitroso derivatives at both pH ranges examined.

Group 5 Products (C-Nitroso and Nitro Derivatives of Drugs)—The highly reactive antipyrine (XX) formed the 4-nitro derivative quite readily in both pH ranges studied. The formation of the C-nitroso derivative of XX was observed only when the reaction was run in 10% acetic acid at room temperature. Aromatic mononitro and dinitro derivatives of drugs containing aromatic rings (IV, XV, XVII, and XIX) were present (yield of 1–5%) in the ethyl acetate extracts of the drug–nitrite reaction mixtures (pH 1–2) as determined from their mass spectra. The location of the nitro groups in the aromatic rings remains to be assigned.

All *N*-nitroso compounds listed under Groups 1 and 2 are known carcinogens (1, 2, 12–14), but the toxicity of the products listed in Groups 3–5 remains unknown. In preliminary studies with tripelennamine (XVI), *N*-nitrosation did not enhance the toxic potential of the drug in rats and the *N*-nitroso derivative was devoid of both central nervous system and antihistaminic activities of the parent drug (8). There is evidence that nitrite has a short half-life in the stomach (14, 23) and that ascorbic acid and other dietary constituents are inhibitors of drug–nitrite reactions (24–27). Nevertheless, the formation of *N*-nitrosamines in the human stomach has been demonstrated (28–30).

Further studies are needed to assess the potentially adverse effects of drug–nitrite interactions in humans, especially those on chronic medication where readily nitrosatable drugs and high dietary intake of nitrite may be involved.

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Application of Mixed Electron-Impact–Chemical Ionization High-Resolution Mass Spectrometry to a Medicinal Agent

Keyphrases □ Structure determination—application of mixed electron-impact–chemical ionization high-resolution mass spectrometry □ Mass spectrometry, mixed electron impact–chemical ionization—structure determination

To the Editor:

Chemical ionization mass spectrometry has proven to be a useful technique for molecular structure determination of various classes of medicinal agents, especially for compounds that contain no molecular ion in the electron-impact spectra. Successful identification of drug metabolites and multicomponent drug mixtures, such as those received by forensic laboratories, is an excellent example of the utility of chemical ionization mass spectrometry. Several comprehensive listings of chemical ionization and electron-impact mass spectra are available for comparison with spectra of unknown drugs (1–3). Therefore, it is useful to obtain both types of spectra for structure elucidation of medicinal agents.

Chemical ionization mass spectra are produced *via* gaseous ion–molecule reactions occurring in the ion source of the mass spectrometer. For an ion–molecule reaction to take place, it is necessary to raise the low pressure (10^{-6} torr) which is characteristic of the conventional electron-impact source. "Closing" the source allows the pressure to increase to approximately 0.5 torr when a reagent gas such as isobutane is added. However, this modification, which facilitates ion–molecule reactions in the chemical ionization mode, may precipitate reactions in the source between sample ions and sample molecules in the electron-impact mode (*i.e.*, self-ionization).

Many investigators have noted the occurrence of collision-induced ions in the electron-impact spectra obtained from closed source instruments (4), particularly MH^+ and fragments generally associated with chemical ionization spectra. Collision-induced ions together with those ions resulting directly from electron impact constitute what is referred to here as a mixed electron-impact–chemical ionization spectra. The ratio of collision ions to electron-impact ions contained in a mixed electron-impact–chemical ion-

Table I—Mixed Electron-Impact–Chemical Ionization High-Resolution Mass Spectral Data of Meprobamate

Elemental Composition	Theoretical	Found	Relative Intensity, %
$C_9H_{19}N_2O_4$	219.1344	219.1335	79
$C_8H_{16}NO_2$	158.1180	158.1177	100
$C_7H_{14}NO_2$	144.1024	144.1007	81
$C_7H_{14}O$	114.1044	114.1032	15
C_7H_{12}	96.0938	96.0931	22
C_6H_{11}	83.0860	83.0844	90
CH_4NO_2	62.0241	62.0242	35
C_4H_7	55.0546	55.0550	68
CH_2NO	44.0136	44.0125	51